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Leading article

Lymphocyte and macrophage interleukin receptors in inflammatory bowel disease: a more selective target for therapy?

The pathogenesis of ulcerative colitis and Crohn's disease involves an interaction between genetic and environmental factors. Whatever the precise mechanism responsible for initiating and perpetuating intestinal inflammation, there is ample evidence for an intense local mucosal immune response, associated with recruitment and activation of lymphocytes and macrophages. The subsequent release of soluble cytokines and other inflammatory mediators causes tissue damage, and contributes to many of the clinical features of these diseases (such as diarrhoea, fever, weight loss, and hypoalbuminaemia) and to the amplification and perpetuation of the local immune response. Whether this is simply an appropriate response to an antigenic challenge (for example a dietary or bacterial antigen entering the mucosa as a result of a primary epithelial barrier defect) or the result of a primary regulation abnormality of the local immune response is not known.[1]

We discuss the evidence of activation of circulating and intestinal lymphocytes and macrophages in inflammatory bowel disease, and examine the role of interleukins (IL) 1 and 2 and the therapeutic potential provided by specific targeting of antagonists to their receptors.

T lymphocyte activation and IL-2

To mediate important regulatory as well as effector immune functions, T cells require to change from a resting to an activated state. This activation is initiated by the interaction of appropriately processed and presented antigen with the T cell receptor. Once activated, the T cell produces a variety of cytokines, including IL-2 which acts as a growth factor for other T cells, induces T cell cytotoxicity, and induces proliferation and differentiation of antibody producing B cells.[2] To exert its biological effect, IL-2, must in turn interact with specific T cell IL-2 receptors (IL-2r). These are not present on resting T cells but are rapidly expressed after interaction with antigen or mitogen. Binding of IL-2 then induces the generation of specific regulatory and effector cells, by stimulating T cell proliferation, differentiation, and clonal expansion of mitogen and antigen reactive cells. Both IL-2 synthesis and IL-2r expression are transient events and the decline in the production of these proteins plays an important role in the normal termination of the T cell immune response. Thus, the interaction of appropriately presented antigen with its T cell receptor confers specificity for an immune response, and the interaction of IL-2 with its receptor determines its magnitude and duration.[3]

The human IL-2 receptor is composed of at least two subunits (α and β), each capable of binding IL-2 with low and intermediate affinities respectively. The association of these subunits results in a high affinity receptor, which is required for the development of an IL-2 dependent proliferative response. The α subunit is inducible on the membrane of activated T cells, B cells, and monocytes.[3] Resting T cells do not express either subunit but when activated by mitogen there is raised expression of both subunits.[4] The rapid rate of association between IL-2 and the α subunit and the inducibility of the α subunit gene contribute to the highly regulated and transient display of high affinity IL-2r on activated T cells.[5]

IL-2 produced by activated T lymphocytes may have an important role in acute and chronic inflammatory disease, including inflammatory bowel disease, by activating T cells, B cells, and macrophages to produce a variety of cytokines.[6] The enhanced release of these cytokines is thought to amplify the immune response by recruiting neutrophils and monocytes to the lesion and by stimulating their activation and proliferation. This, in turn, may contribute to mucosal damage by release of oxygen radicals, proteases, and cytokines.[7] The production of IL-2 by blood and mucosal lamina propria T cells has therefore been investigated. Raised mucosal IL-2 activity was noted after stimulation of both normal and inflamed mucosa, and IL-2 production was reduced by drugs used in the treatment of inflammatory bowel disease (5 amino salicylic acid, corticosteroids, and cyclosporin A). Despite T cell activation (as shown by the presence of αIL-2r), the amount of IL-2 released per lamina propria cell was not raised in inflammatory bowel disease.[7] Bioassay studies have failed to show an increase in IL-2 production by peripheral blood mononuclear cells in active Crohn's disease,[8] or from cultured lamina propria mononuclear cells.[9] One explanation is the immunosuppressive effect of steroid treatment, which reduces IL-2 gene expression,[7] and also malnutrition, which depresses cell mediated immunity.[10] Detectable IL-2 reflects the dynamic balance between IL-2 production and its absorption and internalisation by cells expressing the IL-2r. Since the density of low affinity αIL-2r increases during immune stimulation, this may function to down-regulate the T cell

response by trapping free IL-2.[11] Bioassay data should be interpreted with caution since they are vulnerable to confounding effects of inhibitory molecules and synergistic effects of more than one cytokine.[12] A recent study suggested that reduced IL-2 detection was caused by reduced production by CD4 positive T cells rather than increased absorption of soluble IL-2 inhibitors. Most patients, however, were on steroid treatment.[13]

Two recent studies showed increased IL-2 production. The first study, using ELISA to measure IL-2, showed that its release from sonicated endoscopic biopsy specimens from both Crohn's disease and ulcerative colitis patients was increased compared with controls.[14] The other study detected raised IL-2 mRNA in intestinal mucosa of active Crohn's disease but not in inactive disease of ulcerative colitis.[15]

Since T cell activation is a powerful effector mechanism in chronic inflammation, studies have investigated whether circulating and mucosal T cells are activated in inflammatory bowel disease, as defined by the presence of activation antigens such as αIL-2r.

Circulating T cell activation

The expression of an early activation antigen (T9), on circulating lymphocytes was raised in Crohn's disease, correlating with the Crohn's disease activity index (CDAI) clinical activity score,[16] but activation antigens were not expressed when macroscopic disease had been surgically removed[17] or in quiescent disease,[18] suggesting that the source of activated T cells is the inflamed mucosa. Raised T9 expression was also found in ulcerative colitis but not in acute bacterial or viral enteritis.[19] Thus, circulating lymphocyte T9 expression may offer a simple measure of mucosal disease activity in inflammatory bowel disease.

The development of fluorescence activated cell sorter (FACS) scan analysis now permits quantitation of both the percentage of specifically identified cells expressing a given antigen and also the fluorescent intensity, which gives a measure of cell surface receptor density.[20] Studies using this technique have shown an increased percentage of circulating T cells expressing αIL-2r in active mixed inflammatory bowel disease,[21] in active Crohn's disease, but not ulcerative colitis.[22] and increased αIL-2r density in Crohn's disease which was directly proportional to clinical disease activity measured by the CDAI.[23] Lymphocytes with high density of αIL-2r expression correspond with the S phase of the cell cycle and thus with proliferation.[24] Raised αIL-2r density is also seen in patients with ulcerative colitis but this is less marked (MJW unpublished observations).

Although studies on circulating lymphocytes may be of limited relevance to mucosal events in inflammatory bowel disease,[25] observations on circulating lymphocytes seem to correlate with the degree of mucosal T cell activation[17] as well as disease activity in Crohn's disease.[16 23] Moreover, the study of circulating lymphocytes and monocytes in whole blood avoids the potential for artefact and distortion of lymphocyte subtype proportions which may result from mucosal cell isolation and density gradient separation techniques.[26]

Mucosal T cell activation

Evidence for T cell activation in Crohn's disease mucosal tissue sections was found using a sensitive immuno-alkaline phosphatase technique showing αIL-2r expression on CD4 positive helper T cells in the lamina propria and submucosa but not in normal bowel. In ulcerative colitis, αIL-2r expression was mainly present on macrophages.[27] Using FACS analysis, isolated lamina propria mononuclear cells showed a marked increase in the number of lymphocytes expressing activation antigens in both ulcerative colitis and Crohn's disease,[28] and in another study of ulcerative colitis patients, mucosal T cell αIL-2r and HLA-DR expression were raised.[29] A further study using isolated mucosal cells, however, showed that although many lamina propria T lymphocytes were activated, as shown by expression of HLA-DR and αIL-2r antigens, this finding was not different to controls.[26] These discrepant results may have resulted from technical difficulties relating to the cell isolation procedure which can cause selective subset loss or artefactual activation. Most studies have found evidence of mucosal T cell activation in both ulcerative colitis and Crohn's disease with a varying degree of macrophage αIL-2r expression. Experimental T cell activation in human fetal small intestine produced mucosal destruction similar to that seen in Crohn's disease and resulted in increased HLA-DR and αIL-2r expression on lamina propria macrophages suggesting that primary T cell activation is an important effector mechanism in Crohn's disease. Interferon gamma release from such activated T cells could result in the αIL-2r expression seen on macrophages in inflammatory bowel disease.[30]

Whatever the initial event responsible for such T cell activation, be it infectious or autoimmune, there is evidence that once activated, release of interferon gamma from these cells can result in HLA-DR expression on epithelial cells enabling them to act as antigen presenting cells, resulting in further T cell activation.[31]

Monocyte and macrophage activation in inflammatory bowel disease

Macrophages are a prominent feature of the inflammatory infiltrate in both ulcerative colitis and Crohn's disease. They are important in providing the first line of defence against micro-organisms or toxins that breach the epithelial barrier, by presenting antigen to sensitised T cells and releasing a variety of cytokines and inflammatory mediators.

Circulating monocyte activation

Since mucosal macrophages are probably derived from circulating monocytes,[32 33] several studies have investigated circulating monocyte function in inflammatory bowel disease. There is an increase in monocyte turnover due to increased demand from the inflamed bowel.[32] Circulating monocytes in inflammatory bowel disease are activated, as assessed by a variety of functional measures,[34 35] but the cause of this is unclear. It is unlikely to be a primary phenomenon since this would not explain the characteristic disease localisation in Crohn's disease and ulcerative colitis but may occur in response to luminal antigen or endotoxin absorbed from the gut, just as circulating monocytes and probably activated in response to mycobacterial antigen in pulmonary tuberculosis.[36]

Resting monocytes constitutively express the βIL-2 receptor[4] but do not express the αIL-2 receptor which is needed to form the high affinity IL-2r complex. Monocytes activated with lipopolysaccharide or interferon gamma do, however, express functional αIL-2 receptor.[37] Circulating monocytes in tuberculosis are also activated and express functional αIL-2 receptor.[36] Since there is much evidence for functional activation of circulating monocytes in inflammatory bowel disease, evidence for αIL-2r expression has been sought using FACS analysis but was not

detected.[23] It is not clear whether the absence of detectable αIL-2r on circulating monocytes in inflammatory bowel disease is due to a low receptor density or whether activated αIL-2r bearing monocytes are rapidly removed from the circulation. Alternatively, circulating monocytes in inflammatory bowel disease, although showing some features of preactivation do not express αIL-2r until arrival in the gut mucosa.

Mucosal macrophage activation

Mucosal macrophage αIL-2r expression was not seen in normal bowel mucosa but has been shown in both ulcerative colitis and Crohn's disease.[27 38] In Crohn's disease this is confined to the superficial parts of the mucosa.[27] The mechanism for macrophage αIL-2r expression can be induced by bacterial products such as endotoxin and also by interferon gamma, which is released locally by activated T cells.[37]

The importance of macrophage αIL-2r is unclear. The level of expression of αIL-2r on macrophages is only 10–20% of that found on activated T cells.[37] In vitro studies, however, show that these cells can respond to IL-2, not by proliferation (as do activated T and B cells expressing αIL-2r) but by further activation as shown by inducing oxygen radical release,[37] augmentation of cytotoxicity,[39] and increased IL-1 production.[40] This sequence provides a mechanism whereby macrophage αIL-2r expression may be important in amplification of the immune response. A further possible role for macrophage αIL-2r expression is to down-regulate T cells by competing for IL-2, and thus reducing its availability to high affinity T cell IL-2 receptors.

There is good evidence, besides the finding of αIL-2r expression to suggest that macrophages recruited to the mucosa in inflammatory bowel disease are activated. Macrophages isolated from the colonic mucosa have enhanced respiratory burst activity and antigen presenting activity,[33 38] and ability to release lysozyme and IL-1.[14 33 35] In situ hybridisation technique have also shown increased mRNA for macrophage secretory products including IL-1 in colonic biopsy specimens from patients with inflammatory bowel disease.[41]

IL-1, a polypeptide produced predominantly by stimulated macrophages and monocytes, is an important mediator of pathophysiological events in various acute and chronic inflammatory diseases. It belongs to a group of cytokines, including tumour necrosis factor (TNF) and IL-6, with a wide range of functions relevant to inflammatory bowel disease, and is thought to be an important second signal in T and B cell activation and differentiation. It is one of the first and most prominent molecules synthesised during the acute phase response causing fever, enhancing fibroblast collagen production, and hepatocyte synthesis of acute phase proteins.[12] It also acts as a chemotactic factor for B and T cells, stimulates gene expression for itself as well as for TNF and a number of other interleukins, indirectly promotes chemotaxis of neutrophils and monocytes, causes degranulation of leukocytes, and stimulates synthesis of prostaglandin E2.

Soluble IL2r in inflammatory bowel disease

Activated T cells and macrophages not only express αIL-2r on the cell surface but also release a soluble form of the receptor (sIL-2r).[42] Soluble receptor shedding is a well known phenomenon in cellular biology and has been shown for a number of cytokines including TNF, IL-1, and IL-4. It may represent an important inhibitory mechanism for all of these molecules.[43] Soluble IL-2r is raised in the serum of patients with Crohn's disease,[44–46] and to a lesser extent in ulcerative colitis[47] as well as in a number of other conditions characterised by cellular immune activation such as coeliac disease (in which levels fall on a gluten free diet[48]) and rheumatoid arthritis.[49] Levels of sIL-2r in Crohn's disease[44 46 47] and rheumatoid arthritis[49] correlate well with disease activity. Thus, sIL-2r is a useful marker of immune activation in conditions characterised by T cell activation which may be more specific than acute phase proteins.

The cellular source of sIL-2 receptor in inflammatory bowel disease is unknown. Secretion of sIL-2r by activated peripheral blood mononuclear cells may contribute.[22 47] It is likely, however, that activated T cells and macrophages in the mucosal lesion are the main source.[14 27 44]

The role of sIL-2r is unclear but since it can bind IL-2 and compete with T cells for available IL-2, it has been postulated that it may serve an immunoregulatory function helping to curtail the immune reaction by reducing IL-2 availability.[42] In both rheumatoid arthritis and inflammatory bowel disease, raised sIL-2r is associated with functional inhibition of IL-2 driven responses.[9 49] The inhibitory effect of sIL-2r may be limited, however, since it binds IL-2 with low affinity and was unable to inhibit IL-2 dependent T cell proliferation in vitro.[50] The recently described soluble form of the beta subunit of the IL-2r with higher affinity may have a more important immunoregulatory role.[51]

Potential therapeutic options

Currently available treatments for ulcerative colitis and Crohn's disease depend on non-specific anti-inflammatory effects. 5-ASA and immunosuppressive drugs (steroids or azathioprine) suppress a wide variety of effector mechanisms and induction of remission is associated with inhibition or decrease in the formation of inflammatory mediators. Corticosteroids remain the most effective treatment for inflammatory bowel disease, but have significant dose related toxicity and are ineffective in some patients, who will require surgery. Cyclosporin, a new and more potent immunosuppressant has been evaluated in inflammatory bowel disease. It has been shown to inhibit IL-2 production[52] and αIL-2r expression[53] by T cells. Preliminary studies have shown benefit in both severe active ulcerative colitis[54] and steroid resistant Crohn's disease,[55] and the response was also associated with falling levels of sIL-2r.[45] Its use, however, may also be limited by unacceptable toxicity.[56]

Greater understanding of the pathogenesis of inflammatory bowel disease, and in particular of the central role of activated T cells, has prompted a search for drugs that may inhibit cytokine formation or, alternatively, block their receptors. The goal is to provide more effective and less toxic therapy by developing treatments targeted to specific, cellular effector mechanisms.

Il-1 receptor targeted treatment

Since IL-1 seems to play an important role in the pathogenesis of inflammatory disorders, efforts are being made to modulate interaction with its receptor. A naturally occurring receptor antagonist of IL-1 (IL-1ra) is produced by the same cells (monocytes and macrophages) that are the main source of IL-1. The molecule has been cloned and recombinant human IL-1ra expressed by *Eschencia coli* is effective in competitively blocking binding of Il-1 to its receptor.[57] The local balance of IL-1 and IL-1ra seems to influence the variable clinical activity of acute inflammatory arthritis,[58] and

loss of IL-1 inhibition seems to correlate with clinical relapse in Crohn's disease.[59]

IL-1ra treatment has been tested in an animal model of colitis. Immune complex colitis induced in rabbits results in the production of raised tissue concentrations of IL-1 which correlates with the degree of inflammation and subsequent production of leukotriene, B_4, which is also thought to play a part in the pathogenesis of ulcerative colitis.[60] Pretreatment of rabbits with IL-1ra resulted in a considerable decrease in tissue inflammation and necrosis.[61] This encouraging protective effect suggests that Il-1ra may have a place in the treatment of human inflammatory bowel disease. It remains to be seen, however, if this approach will be as effective in established disease. The mechanism of action may involve reduced IL-1 induced expression of endothelial cell adhesion molecules and IL-8 production thus reducing chemotaxis,[12] rather than inhibition of T cell activation, since complete inhibition of IL-1 binding to T cells using this antagonist in vitro does not prevent mitogen or antigen induced T cell proliferation.[62]

IL-2 receptor targeted treatment

While the role of cytokine inhibitors, such as IL-1ra, in the treatment of inflammatory bowel disease needs evaluation, potentially greater benefit may derive from targeting T cells which produce a variety of cytokines and play a key role in initiating the inflammatory cascade. T lymphocyte depletion may be of benefit in treatment of patients with steroid resistant Crohn's disease.[63] The recent report of the onset of complete remission in a patient with severe Crohn's disease as CD4 counts fell as a result of HIV infection underlined the important role of CD4 helper T cells in the pathogenesis of this disease.[30 64] (A similar phenomenon has recently been described in rheumatoid arthritis.[65]) This raises the possibility that depletion of specific lymphocyte subsets may be useful in treatment. Monoclonal antibodies against CD4 have been used successfully in treatment of inflammatory bowel disease in a pilot study.[66]

Anti-CD4 treatment would target all helper T cells including resting cells, thus potentially inducing appreciable immunosuppression. More selective targeting of activated T cells is therefore needed. Since activated T cells in inflammatory bowel disease, allograft rejection, and various autoimmune disorders express αIL-2r whereas normal resting T cells do not, antibodies to this receptor would provide such selectivity.[3 11] Initial studies using a mouse derived αIL-2r antibody detected a host anti-mouse immune response but a 'humanised' form is now available which is free of this problem.[69] The use of αIL-2r antibodies has shown benefit in experimental animal studies of autoimmune diabetes mellitus and systemic lupus erythematosis.[43] Prophylactic use of these antibodies is effective in preventing renal allograft rejection after transplantation.[3 67] Clinical trials have been initiated in patients with various autoimmune disorders as well as after transplantation, and anti-IL-2r antibodies are well tolerated. A preliminary report also described a rapid response in refractory rheumatoid arthritis.[68] This approach may be particularly beneficial in resistant cases of Crohn's disease in which a dense mucosal infiltrate of T cells bearing αIL-2 receptors is seen.[30]

Finally, the discovery of soluble IL-1 and IL-2 receptors may offer further therapeutic possibilities. Since one of the proposed roles of such receptors may be to down-regulate the immune response by reducing cytokine availability, they have the potential to act as immunosuppressants. The potent immunosuppressant activity of a soluble IL-1 receptor has been shown in animal models of autoimmunity and graft rejection and clinical trials are planned.[70]

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